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本論文（Full paper）

Estimation of hemolymph CO_2 solubility coefficient for acid-base balance in *Pinctada fucata martensii Crassostrea gigas* and *Mimachlamys nobilis*

Takeshi Handa† and Akira Araki

Abstract: The influence of temperature on the hemolymph CO_2 solubility coefficient ($\alpha\mathrm{co}_2$, μM/L/torr) was investigated in the marine bivalves, akoya pearl oyster *Pinctada fucata martensii*, Pacific oyster *Crassostrea gigas* and noble scallop *Mimachlamys nobilis*. Hemolymph samples were equilibrated with standard CO_2 gas mixtures to obtain expressions for $\alpha\mathrm{co}_2$ as a function of temperature. Relationships between hemolymph $\alpha\mathrm{co}_2$ and temperature (T) were expressed as follows: $\alpha\mathrm{co}_2 = 76.88145 - 2.62637 \cdot T + 0.036203 \cdot T^2$ (for *P. fucata martensii*), $\alpha\mathrm{co}_2 = 94.2109 - 3.6252 \cdot T + 0.0554 \cdot T^2$ (for *C. gigas*), $\alpha\mathrm{co}_2 = 70.4591 - 1.5253 \cdot T + 0.0103 \cdot T^2$ (for *M. nobilis*). In the distribution of hemolymph $\alpha\mathrm{co}_2$ values, there was no statistical significance across an interspecies comparison of $\alpha\mathrm{co}_2$. From this result, the regression was fitted using all values of $\alpha\mathrm{co}_2$ and temperature across the species, and the relationship was expressed as follows: $\alpha\mathrm{co}_2 = 182.3717 - 24.3932 \cdot T + 1.6396 \cdot T^2 - 0.0492 \cdot T^3 + 0.000536 \cdot T^4$. This polynomial is versatile equation and would yield the hemolymph $\alpha\mathrm{co}_2$ at arbitrary temperature, even with different species.

Key words: *Pinctada fucata martensii*, *Crassostrea gigas*, *Mimachlamys nobilis*, temperature, CO_2 solubility ($\alpha\mathrm{co}_2$), hemolymph acid–base balance

Introduction

In Bivalvia, the shell valve formation (calcium carbonate crystal) is closely related to respiration, CO_2 dynamics, and hemolymph acid–base balance. Marine bivalve hemolymph generally has a very low CO_2 partial pressure ($P\mathrm{co}_2$) under normal conditions, and around 0.8-2.2 torr under normoxic and normocapnic conditions[1-15]. These $P\mathrm{co}_2$ values were calculated using the Henderson–Hasselbalch equation. As measurement of low $P\mathrm{co}_2$ values is difficult for small hemolymph volumes, hemolymph $P\mathrm{co}_2$ is usually calculated via the Henderson–Hasselbalch equation when evaluating respiratory physiology and acid–base balance. Boutilier et al.[16] noted that $P\mathrm{co}_2$ estimation by application of the Henderson-Hasselbalch equation is widely practiced in studies of fish blood acid–base balance owing to the relative ease and accuracy with which plasma total CO_2 and blood pH may be measured for small volumes. Such the estimates require known CO_2 solubility coefficient ($\alpha\mathrm{co}_2$) and the apparent dissociation constant of carbonic acid[16], and those values of the hemolymph were strongly affected with temperature in hard-shelled mussel *Mytilus coruscus*[17]. The $\alpha\mathrm{co}_2$ is directly related to the calculation of the dissociation constant of carbonic acid and bicarbonate ion concentration, and is considered the most important parameter.

In this study, we analyzed $\alpha\mathrm{co}_2$ of the hemolymph which were collected from the akoya pearl oyster *Pinctada fucata martensii*, Pacific oyster *Crassostrea gigas* and noble scallop *Mimachlamys nobilis*, and obtained the relational expression over the different species at various temperatures.

Materials and Methods

Experimental animals and conditions

These experiments used the akoya pearl oyster, Pacific oyster and noble scallop. Akoya pearl oysters (n = 36;

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mean wet weight, 33 g) and noble scallops (n = 26; mean wet weight, 102 g) were obtained from marine farms in Nagasaki Prefecture, and Pacific oysters (n = 31; mean wet weight, 120 g) were obtained from a marine farm in Hiroshima Prefecture. After cleaning their shell valves, bivalves were reared for a year with seasonal change of water temperature (10–31°C), and fed cultivated phytoplankton[18-20]. Twentyfour hours before hemolymph collection, bivalves were transferred to particle-free (> 0.45 μm) seawater at each experimental temperature. All experiments were conducted in seawater with a salinity of 30 psu, O_2 saturation 90–95%, pH7.9–8.1, and a total CO_2 concentration of 1.4–1.8 mM/L.

Hemolymph collection and analysis

Adductor muscle hemolymph was anaerobically collected by direct puncture using a gas-tight microsyringe (Model 1750LTN, Hamilton), obtaining approximately 0.2–0.4 mL of hemolymph. The hemolymph was adjusted to pH2.5 with lactic acid (Wako Pure Chemical Industries, Ltd.) for *in vitro* αco_2 evaluation. Acidified samples were transferred to a tonometer flask and equilibrated with humidified standard CO_2 gas (CO_2, 5.0-15%; O_2, 20.9%; N_2 Balance) using an equilibrator (DEQ-1, Cameron Instruments Co., Texas) at experimental temperature (9.7–30.6°C). After equilibration, sample total CO_2 concentration (Tco_2) was measured using a total CO_2 analyzer (Capnicon 5, Cameron Instruments Co.). Pco_2 values were calculated using the known standard gas CO_2 concentration, barometric pressure, and water vapor pressure. The following expression was used to calculate αco_2:

$$\alpha co_2 = Tco_2 \cdot Pco_2^{\ -1}$$

where units used are μM/L/torr for αco_2, μM/L for Tco_2, and torr for Pco_2.

Statistical analysis

Distribution normality of hemolymph properties was assessed using the Shapiro–Wilk test. The Kruskal–Wallis test was performed for hemolymph αco_2 fluctuation with temperature change across each species, and for hemolymph αco_2 distribution among the three species. Statistically significant differences were set at $P < 0.05$. All analyses were conducted using the statistical software Kyplot 6.0 (KyensLab Inc., Japan).

Results and Discussion

The influence of temperature on hemolymph αco_2 values for akoya pearl oyster, Pacific oyster, and noble scallop were investigated, and the relationship clarified. The mean αco_2 for akoya pearl oyster was 30.4 − 53.5 μM/L/torr between 10.4°C and 30.6°C (Fig. 1). Pacific oyster yielded an αco_2 of 34.6 − 65.6 μM/L/torr between 9.7°C and 29.8°C (Fig. 2). And the αco_2 of noble scallop were 32.7 − 48.8 μM/L/torr between 16.6°C and 30.3°C (Fig. 3). Cameron[21)] reported CO_2 solubility as a function of temperature and salinity, with solubility coefficients of 33.01 − 69.78 mM/L/torr at a salinity of 30 between 6°C and 32°C. The obtained hemolymph αco_2 reflected that reported by Cameron[21)]. Although the information with respect to the αco_2 of marine bivalves is limited, the hemolymph αco_2 decreased with increasing temperature for akoya pearl oyster, Pacific oyster, and noble scallop ($P < 0.05$, Kruskal–Wallis test). A similar response was reported for the hemolymph of the hard-shelled mussel between 16°C and 28°C[17)].

For akoya pearl oyster hemolymph, an equation was fitted to the αco_2 data within the temperature range (Fig. 1). The relationship between αco_2 and temperature is expressed as follows:

(Akoya pearl oyster hemolymph)

$$\alpha co_2 = 76.88145 - 2.62637 \cdot T + 0.036203 \cdot T^2 \ (R^2 = 0.999)$$

where T is the temperature, and units used are μM/L/torr for αco_2 and °C for T.

The relational expressions of other experimental animals, Pacific oyster and noble scallop (Figs. 1, 2), are similarly shown as follows:

(Pacific oyster hemolymph)

$$\alpha co_2 = 94.2109 - 3.6252 \cdot T + 0.0554 \cdot T^2 \ (R^2 = 0.965)$$

(Noble scallop hemolymph)

$$\alpha co_2 = 70.4591 - 1.5253 \cdot T + 0.0103 \cdot T^2 \ (R^2 = 0.953)$$

where T is temperature, and units of μM/L/torr are for αco_2 and °C for T. These relational expressions estimate the coefficient at arbitrary temperatures for each species.

The distribution of hemolymph αco_2 values for akoya pearl oyster, Pacific oyster, and noble scallop are shown in Fig. 4. In the distribution of hemolymph αco_2 values, no statistical significance was observed across an interspecies comparison of αco_2 ($P > 0.05$, Kruskal–Wallis test). From this distributional result, the regression was fitted using all values of αco_2 and temperature across the species evaluated (Fig. 4), yielding the following relationship between αco_2 and temperature:

$$\alpha co_2 = 182.3717 - 24.3932 \cdot T + 1.6396 \cdot T^2 - 0.0492 \cdot T^3 + 0.000536 \cdot T^4 \quad (R^2 = 0.805)$$

where T is temperature in °C.

When verifying this relationship by calculating αco_2, values estimated were 58.6 mM/L/torr at 10°C, 39.0 mM/L/torr at 24°C, and 36.7 mM/L/torr at 26°C, respectively. These are consistent with hemolymph αco_2 values obtained previously; 59 μM/L/torr for Pacific oyster at 10°C, 39 μM/L/torr for noble scallop at 24°C, and 37 μM/L/torr for akoya pearl oyster at 26°C, respectively[7,11,13]. The polynomial regression developed in this study yields αco_2 values reflecting those obtained by hemolymph analysis. Temperature and salinity both influence αco_2 values[21]. Hevert[22] and Eble[23] described Pacific oysters to be osmotic conformers over the range 902 to 1,358 mM/kg. In this study, osmotic pressure was not measured, but as marine bivalves are generally osmotic conformers[24], it is assumed that there is little difference in hemolymph osmotic pressure among the different species in this study. Therefore, the polynomial regression developed here conveniently obtains αco_2 at arbitrary temperatures, even with different species. The three species in this study, akoya pearl oyster, Pacific oyster and noble scallop, are belonging to subclass Pteriomorphia (= Autolamellibranchiata)[25]. This subclass contains many usefulness species in Japan marine bivalves, for example, the ark shell (Order Arcoidea) and mussel (Order Mytiloidea) are also contained in Pteriomoriphia[25]. Combining additional αco_2 values of those species would further improve the accuracy of the polynomial equation.

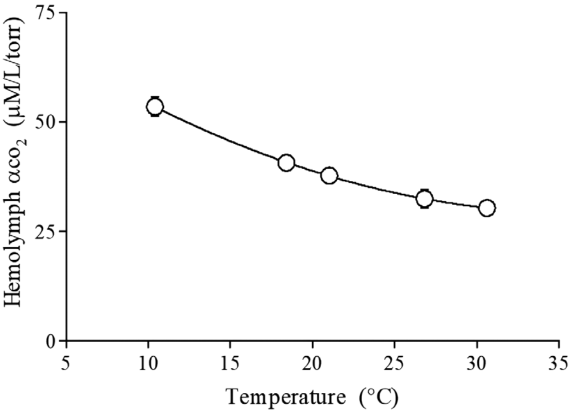

Fig. 1. Influence of temperature on hemolymph CO_2 solubility coefficient (αco_2) for akoya pearl oyster *Pinctada fucata martensii*. Data are mean and standard deviation (n = 6-11 in each). The solid line is fitted to the data and the equation: $\alpha co_2 = 76.88145 - 2.62637 \cdot T + 0.036203 \cdot T^2$ ($R^2 = 0.9999$).

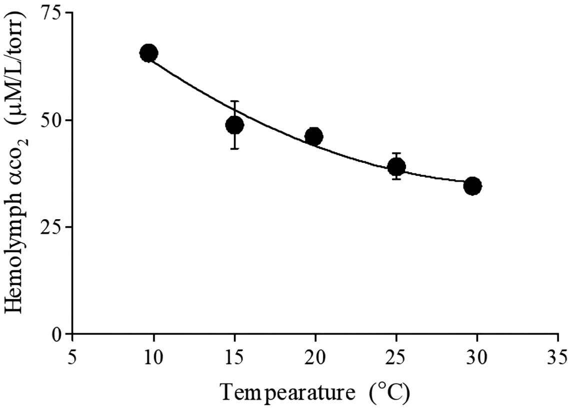

Fig. 2. Influence of temperature on hemolymph CO2 solubility coefficient (αco_2) for Pacific oyster *Crassostrea gigas*. Data are mean and standard deviation (n = 5-7 in each). The solid line is fitted to the data and the equation: $\alpha co_2 = 94.21085 - 3.62519 \cdot T + 0.055408 \cdot T^2$ ($R^2 = 0.9649$).

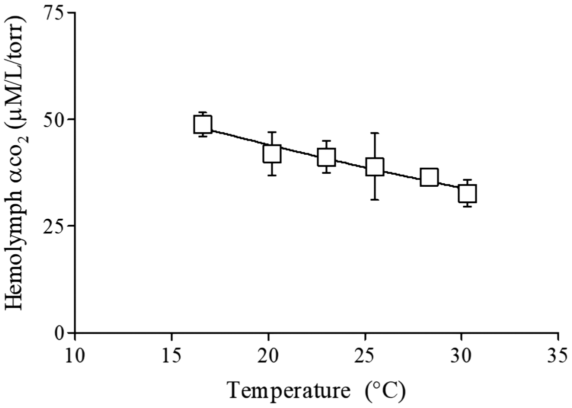

Fig. 3. Influence of temperature on hemolymph CO_2 solubility coefficient (αco_2) for noble scallop *Mimachlamys nobilis*. Data are mean and standard deviation (n = 3-7 in each). The solid line is fitted to the data and the equation: $\alpha\text{co}_2 = 70.45912 - 1.52531 \cdot T + 0.010257 \cdot T^2$ ($R^2 = 0.9534$).

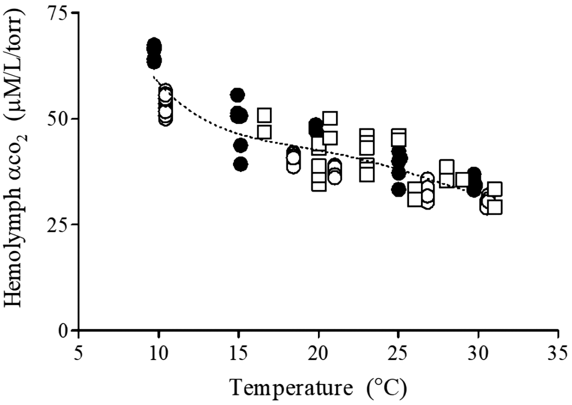

Fig. 4. Influence of temperature on hemolymph CO_2 solubility coefficient (αco_2) for akoya pearl oyster *Pinctada fucata martensii* (open circle, n = 36), Pacific oyster *Crassostrea gigas* (closed circle, n = 31), noble scallop *Mimachlamys nobilis* (open square, n = 26). Data are analytical values obtained from *in vitro* experiments. The dotted line is fitted to all data which integrated the different species (n = 93), and indicated the equation: $\alpha\text{co}_2 = 182.3717 - 24.3932 \cdot T + 1.639579 \cdot T^2 - 0.0492 \cdot T^3 + 0.000536 \cdot T^4$ ($R^2 = 0.8052$).

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アコヤガイ，マガキ，ヒオウギの酸塩基平衡における ヘモリンパ液の二酸化炭素溶解度係数の推定

半田岳志，荒木　晶

要旨: アコヤガイ*Pinctada fucata martensii*, マガキ *Crassostrea gigas*，ヒオウギ *Mimachlamys nobilis* から採取したヘモリンパ液を用いて二酸化炭素溶解度係数 (αco_2，μM/L/torr) に及ぼす温度 (T，℃) の影響について調べ，αco_2 とT の関係を明らかにした。アコヤガイで得た関係式は，$\alpha co_2 = 76.88145 - 2.62637 \cdot T + 0.036203 \cdot T^2$ であった。同様にマガキとヒオウギは $\alpha co_2 = 94.2109 - 3.6252 \cdot T + 0.0554 \cdot T^2$ (マガキ)，$\alpha co_2 = 70.4591 - 1.5253 \cdot T + 0.0103 \cdot T^2$ (ヒオウギ) だった。これら３種の αco_2 の分布を比較すると統計的有意差は認められなかったことから，３種の αco_2 と温度の全ての結果に対して多項式回帰を適用し，以下の関係式を得た。$\alpha co_2 = 182.3717 - 24.3932 \cdot T + 1.6396 \cdot T^2 - 0.0492 \cdot T^3 + 0.000536 \cdot T^4$。この式により，種が異なっても，任意の温度で海産二枚貝の αco_2 を容易に得られ，酸塩基平衡，二酸化炭素動態および呼吸生理の評価に有効と考えられる。